Genetic Polymorphism of Cytochrome P450 as a Biomarker of Susceptibility to Environmental Toxicity

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Cytochrome P450 (CYP) enzymes are responsible for the metabolism of numerous xenobiotics and endogenous compounds, including the metabolic activation of most environmental toxic chemicals and carcinogens. Both metabolic and genetic polymorphisms have been identified for human CYP enzymes. The association of CYP genetic polymorphism and human cancer risk, and susceptibility to environmental hazards, have received increasing attention. This article briefly reviews the approaches and methods currently used in CYP genetic polymorphism studies. In addition, the current status and perspectives of using CYP genetic polymorphism as a biomarker of individual susceptibility to cancer and environmental toxicity are discussed. — Environ Health Perspect 105(Suppl 4):759–762 (1997)

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Cytochrome P450s (CYPs) are a superfamily of hemoproteins that catalyze the biotransformation of various xenobiotics and endobiotics (*1,2*). Although there are overlapping substrate specificities, individual CYP forms have significant differences in their substrate preferences. On the basis of this specificity, selective activity assays with different substrates have been used to establish markers for particular CYP forms.

It is well established that most environmental toxic chemicals and carcinogens need to be metabolically activated to exert their toxic or carcinogenic effects. As a major enzyme system in xenobiotic metabolism, CYPs play a critical role in the metabolic activation of many environmental chemicals. In some cases, CYP-catalyzed metabolism leads to the detoxification of toxic chemicals.

Of 15 human CYP enzymes so far characterized, 8 forms of CYP (CYP1A1, 1A2, 2A6, 2C9, 2C19, 2D6, 2E1, and 3A4) have been shown to be polymorphic at the phenotypic or genotypic level, or both (*3–5*). In addition to metabolizing various drugs, these polymorphic CYP enzymes are involved in metabolizing a large number of environmental carcinogens and toxic compounds (*1*). Because an individual's capability to metabolize these toxicants can be altered by carrying the variant alleles, genetic polymorphisms of CYP enzymes have been proposed as a biomarker of susceptibility to environmental carcinogenesis and toxicity. This paper briefly reviews the methodologies for CYP polymorphism studies and emphasizes the comparison between the genotyping and phenotyping approaches. The use of genetic polymorphisms of human CYP enzymes as a susceptibility biomarker and the direction of future research will be discussed.

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Abbreviations used: cDNA, complementary DNA; CYP, cytochrome P450; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; RT–PCR, reverse transcription coupled with PCR.

Phenotypic Determination of CYP Polymorphisms

It is well known that there are large interindividual variations in CYP-catalyzed drug biotransformation (*1*). The CYP polymorphisms were initially identified by determining the metabolic ratio, i.e., the ratio of the blood or urinary amount of the parent drug over its metabolite, in individuals to whom the probe drug was administered. Metabolic polymorphism is usually indicated in a population where the frequency distribution of the metabolic ratio is shown to be bimodal or trimodal. The bimodal distribution is due to the existence of poor and extensive metabolizers, whereas the trimodal distribution is caused by the presence of additional intermediate metabolizers. Examples for bimodal distribution include debrisoquine 4-hydroxylation (catalyzed by CYP2D6) and coumarin 7-hydroxylation (catalyzed by CYP2A6) (*6*). The 3-demethylation of caffeine is an activity marker of CYP1A2, and a trimodal distribution for caffeine metabolism was observed in nonsmokers (*7*).

Besides the metabolism *in vivo*, the polymorphisms of CYP enzymes can be determined at other phenotypic levels. These include enzyme activity determinations for metabolism *in vitro* with microsomes, CYP enzyme protein levels detected by immunological methods such as immunoblot and immunohistochemical analyses, and CYP mRNA levels measured by different nucleic acid hybridization techniques (Northern and slot blotting, RNase protection, and *in situ* hybridization). In recent years researchers have developed reverse transcription coupled with polymerase chain reaction (RT–PCR) to detect CYP mRNA in a small amount of tissue sample. If an appropriate internal standard is included in the cDNA synthesis reaction, the RT–PCR can be quantitative in assessing the amount of CYP mRNA in the samples. Expression of CYP1A1 mRNA in human lymphocytes and its regulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin have been successfully determined by this method (*8*).

The phenotyping approach, especially with activity-related assays such as metabolism *in vivo* and *in vitro*, directly links the expression of a given CYP form to the metabolism of its probe substrate. However, a critical issue is the difficulty in finding the right probe drugs that are specific for diagnosing a particular CYP form and safe enough for *in vivo* metabolism studies. Another major concern with the phenotyping approach is the confounding effects of dietary factors or coadministration of drugs that could affect expression of the metabolizing CYP enzymes (*9*). This could, in turn, alter the metabolism of the probe drug. Induction or suppression of CYP enzyme activities by xenobiotics, including dietary compounds, has been

well documented in animals (*9*). In humans, induction of CYP2E1 by alcohol drinking (*10*) and of CYP1A2 by ingestion of charbroiled meats or cruciferous vegetables have been observed (*11*). In addition, human CYP1A2 is induced by cigarette smoking (*12,13*). Finally, the availability of substantial amounts of quality-assured human tissues (except peripheral blood cells) could be a limiting factor for determination of CYP enzyme activity *in vitro*, as well as for determination of CYP protein and mRNA levels.

Genetic Basis of Metabolic Polymorphisms

The genetic basis of several CYP-involved metabolic polymorphisms has been elucidated. Mutations in the CYP genes are believed to be a major mechanism for altering enzyme expression and/or catalytic activities. Both point mutations and deletions are observed in the polymorphic CYP genes. Depending on their locations in the gene sequence, polymorphic changes could have either two general effects or none at all. Mutations in the coding region of a CYP gene that cause amino acid substitutions could alter the catalytic activity by causing a direct change in the protein structure. Mutations in the noncoding region, on the other side, may alter the level of mRNA expression by influencing transcription, mRNA stabilization, or pre-mRNA splicing. All of the mutations with functional significance are believed to have a remarkable impact on an individual's capability to metabolize certain drugs and environmental chemicals. However, the functional significance of a majority of the polymorphic changes in CYP genes so far identified is still not known.

Once the metabolic polymorphism involving a particular CYP enzyme is demonstrated, various molecular biology approaches and techniques can be used to look for possible genetic changes. A successful example of this approach is the discovery of *CYP2D6* genetic polymorphism by Gonzales et al. (*14*). After cDNA cloning and DNA sequencing, they demonstrated that a mutant *2D6* allele is responsible for the majority of "poor metabolizers." Further work established that a mutation at a splicing site caused the production of defective 2D6 mRNA and a total absence of 2D6 protein (*15*). Prior to labor-intensive DNA sequencing work, the possible DNA sequence alterations can now be screened by single-strand conformation polymorphism analysis that detects the mutation-caused mobility shift of the DNA fragments on gel electrophoresis (*16*) or by other methods such as denaturing gradient gel electrophoresis (*17*). For functional analysis, the catalytic activity of CYP enzymes can be studied by expressing different variant CYP proteins with various cDNA expression systems if the polymorphic changes are localized in the coding region. If the polymorphic loci are in the noncoding region, their effects on the transcriptional regulation can be studied by linking the mutated sequence with a reporter gene.

Genotyping Approach

As long as the polymorphic sites of a CYP gene are clearly identified, it is simple to determine an individual's genotype by current molecular biology techniques. If a polymorphic site in a CYP gene changes the recognition sequence of a restriction enzyme, or if the genetic polymorphism involves a large deletion, the genetic polymorphism can be identified by restriction fragment length polymorphism (RFLP) analysis, in which DNA is subjected to Southern blotting after digestion with appropriate restriction enzymes and hybridized with specific probes.

Recent advances in PCR technology have greatly increased our capability to detect genetic polymorphisms of CYP enzymes. With small amounts of human tissue or cell samples, DNA amplification can be carried out with proper PCR primers for any particular sequence of a polymorphic CYP gene. The DNA source can be from blood leukocytes, buccal epithelial cells, hair roots, or normally exfoliated cells, such as bladder epithelium in the urine. DNA can also be obtained from stored pathological tissue sections, which provide great advantage for retrospective studies. It is now feasible to determine several CYP genetic polymorphisms with less than 10 µl of blood collected from the finger tip. The PCR-amplified DNA sequence containing the polymorphic sites can then be analyzed by RFLP with restriction digestion and visualized on a stained gel after electrophoresis. Comparing the wild-type samples, if the genetic polymorphism results in a loss—or in some cases a gain—of a restriction site, the band pattern on the gel will be different. If the PCR primers are designed to be within the missing sequence of a deletion polymorphism, there will be no PCR product formed.

Obviously, the RFLP method cannot be used to screen the CYP genetic polymorphisms in which the DNA sequence alterations cause no changes at suitable restriction sites. In this case, genotyping can be carried out by allele-specific PCR with a set of mutation-specific primers for amplification. Several polymorphisms of *CYP1A1*, *2A6*, and *2D6* have been identified by the allele-specific PCR method (*18–20*). If necessary, the results from PCR–RFLP and allele-specific PCR can be confirmed by PCR-direct sequencing.

In contrast to the phenotyping approach, genotyping is not affected by drugs or dietary factors that might modulate the metabolic activity of CYP enzymes. As mentioned previously, the PCR-based genotyping techniques require only a small amount of DNA, which can be obtained by less invasive or noninvasive means or from longtime stored pathological samples. The genotyping approach also allows accurate prediction of the homozygous or heterozygous status of an individual who carries the variant allele. All of these are particularly useful for large population studies in which genetic polymorphisms of CYP enzymes may be susceptibility markers.

CYP Genetic Polymorphisms and Cancer Risk

The association of CYP genetic polymorphisms and human cancer risk has received increasing attention. Examples include *CYP1A1* with lung and breast cancers (*21–23*), *CYP2D6* with different types of cancer (*24*), and *CYP2E1* with lung, liver, and nasopharyngeal cancers (*21,25–28*). However, many reports are controversial. One important factor in interpreting these results is that there are significant ethnic differences in frequency distribution of the CYP genetic polymorphisms. For example, an association of *CYP2E1 Dra*I genetic polymorphism and susceptibility to lung cancer was suggested in a study of a Japanese population (*29*) but was not observed in Caucasians (*26,27,30*). This discrepancy was believed to be caused by a significantly low distribution frequency of *CYP2E1 Dra*I polymorphism in Caucasian populations (*27*).

Current Problems and Perspectives

Research on genetic polymorphisms of CYP enzymes can provide a molecular basis for interindividual variations in metabolizing drugs and environmental toxic chemicals. In addition to this mechanistic information, the studies hold great promise in identifying susceptible individuals and protecting them from environmental toxicity. If a given

CYP polymorphic genotype causes enhancement in the metabolic activation of the related substrate toxicants, individuals with such variant alleles should avoid exposure to those toxic compounds. Knowing the identity of the chemical to which one would be exposed and the polymorphic CYP form involved could be particularly useful in preventing chemical toxicity from occupational exposure. Studies on CYP genetic polymorphism and environmental toxicity could be very rewarding, and research activities in this field are expected to increase in the near future. However, we are facing the following challenges:

a) With current PCR-based techniques and rapid development of molecular biology approaches, identification of CYP genetic polymorphisms and genotypic screening of a subpopulation are not difficult. More new CYP genetic polymorphisms are expected to be discovered. The most critical challenge, however, is to establish the functional importance of different polymorphic variants, especially for those polymorphic sites located in the noncoding regions of the CYP genes. Using an *in vitro* transfection system with *CAT* as a reporter gene, it has been observed that the *Rsa*I polymorphic site in the 5′-flanking sequence of the *CYP2E1* gene caused a 10-fold increase in transcriptional activity in comparison with the wild-type sequence (*31*). It is important to demonstrate whether such extent of regulation occurs *in vivo*. Similarly, the results from the *in vitro* activity assays with the expressed variant CYP proteins need to be verified in phenotypic studies with human populations.

b) Our current efforts have been focused on the association of CYP genetic polymorphisms with cancer risks and less on the occupational toxicity in which the biological end points are not cancer occurrence. Human carcinogenesis is a long-term, multistep process. Although metabolic activation by CYP enzymes is known to constitute the first and critical step in environmental carcinogens, there are many other important steps involved, such as phase II enzyme detoxification, DNA repair, and immunosurveillance, as well as many modulating factors such as dietary components. In addition, it is difficult or impossible to know the number and identity of the carcinogens involved or the exposure levels. Assessing the role of genetic polymorphism of a particular CYP form by using cancer occurrence as a biological end point is therefore a very difficult task. We believe that at this stage it is more feasible to determine the role of CYP genetic polymorphisms in the susceptibility of workers to chemical toxicity in occupational exposures in which cancer occurrence is not an end point. An advantage in using worker populations is that the identity and the exposure levels of the toxic chemicals are in general clearly known. In addition, the workers are probably more homogeneous than the general population and more easily accessible for follow-up studies.

c) More research is needed for characterization of the substrate specificity and the enzyme kinetics of human CYP enzymes. Knowledge of which environmental toxicants and carcinogens are the substrates of the polymorphic CYP enzymes is important, but that knowledge is not enough in designing a population study. Although it is known that CYP enzymes have overlapping substrate specificities, the CYP form with a high V_{max} value and the lowest K_m value is usually the principal one involved in the metabolism and is therefore believed to be the one most relevant to the situation *in vivo*. Obviously, if a particular CYP form is found to be able to activate a toxic chemical but the K_m value is much higher than the physiological concentration of that chemical after exposure, that CYP form may be of little relevance to the real *in vivo* situation. Studies in this direction will also help in developing more selective "probe" drugs for different CYP forms, which is inevitably required in establishing the relationship between CYP genetic polymophisms and metabolic activity *in vivo*.

d) More information is needed to understand the regulation of CYP enzymes in humans. The studies should include the effect of dietary compounds on the expression of CYP enzymes and the expression profile of CYP enzymes in the tissues that are targets for chemical toxicity or carcinogenesis. Without this information, the role of CYP genetic polymorphism in the biological consequences may not be accurately assessed.

e) Humans are exposed to numerous environmental toxicants. For example, more than 40 carcinogens have been found in tobacco products and tobacco smoke (*32*). Multiple polymorphic CYP enzymes are involved in metabolizing these carcinogens. Even for a given subpopulation mainly exposed to a single toxic chemical, it is possible that more than one form of CYP enzyme is involved in the activation or detoxification. Therefore, it is recommended that whenever possible a combinational polymorphism analysis on all the involved CYP forms be carried out to obtain a complete picture of the role of CYP enzymes in susceptibility to cancer and toxicity. The availability of current PCR technology allows us to use DNA samples stored for extensive periods (e.g., blood dotted on filter paper or pathological tissue sections) for the analysis of different CYP genetic polymorphisms. In addition, these DNA samples can be used for combinational genetic polymorphism analysis with the phase II metabolizing enzymes such as glutathione *S*-transferase and *N*-acetyltransferase, or DNA repair enzymes.

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